High-resolution subcellular imaging at the ESRF new nanoimaging beamline: deciphering intracellular targets of anticancer drugs in breast cancer cells

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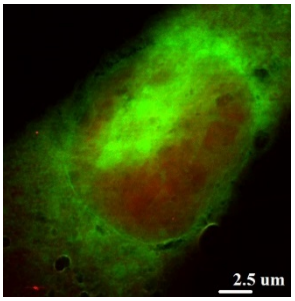
Introduction and Objectives

The new state-of-the-art beamline ID16A-NI at ESRF offers unique capabilities for X-ray imaging at nanometer scale delivering a highly coherent, very intense nanofocused beam (> 5 10^{11} ph/s at $\Delta\lambda/\lambda \sim 10^{-2}$) at high energies (~20nm at 17kev). It is particularly well suited for the investigation of biological samples at high resolution, e.g. the detection and quantification of trace elements [2], such as metals in metal-based drugs in cancer treatment. Triple negative breast cancer tumors, responsible for a high rate of mortality, are a major challenge for breast oncologists since no targeting therapy is currently available for them. Jaouen's group has developed [1] ferrocenyl metal-based drug candidates that can target both hormone-dependent and independent breast cancer cells at low nM range showing encouraging anticancer effects. Our aim is to identify the targeted intracellular compartments where these compounds are active as a main step towards explaining their action mechanisms.

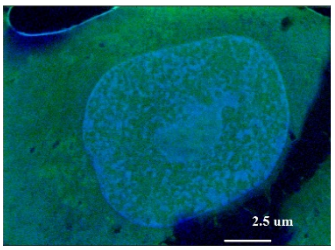
Results and Discussion

Osmium derivatives of the ferrocenyl based drug were imaged in MDA-MB-231 breast cancer cell line using both X-ray fluorescence and phase imaging. Various sample preparation techniques were explored. First chemically fixed cells were imaged in 2D (fluorescence at 50 nm and phase imaging at 10 nm pixel size) to reveal the quantitative elemental distribution. Then we imaged 200 nm thin sections prepared by high pressure freezing for fixation followed by cryo-substitution and resin embedding. Finally X-ray fluorescence tomography scans of the entire cells were performed at 150 nm step size.



Conclusions

The 2D fluorescence maps of entire cells consistently revealed a pattern of high Os concentration alongside the nuclear membrane, localization further confirmed by the 3D fluo-tomography data. This work confirms the potential to reveal structural information at unprecedented resolution.



Fluorescence maps of breast cancer cells at 50 nm pixel size. Up: Zn (red) and Os (green). Down: 200 nm section, Cl (green), P (blue)

References

[1] Hillard, E. A.; Vessieres, A.; Jaouen, G., Ferrocene Functionalized Endocrine Modulators as Anticancer Agents. In *Medicinal Organometallic Chemistry*, Jaouen, G.; Metzler-Nolte, N., Eds. Springer Verlag, 2010; Vol. 32, pp 81-118.

[2] Bohic S. et al., Biomedical applications of the ESRF synchrotron-based microspectroscopy platform, Journal of Structural Biology, 177 (2012) 248-258